In vitro digestibility using caecal liquor of diets containing poor quality roughages and green forages fed to domesticated ostriches (Struthio camelus var. domesticus)

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Abstract

The objective of this study was to compare in vitro organic matter digestibility (IVOMD) of ostrich diets using the normal (NTT) and reverse (RTT) modified Tilley and Terry methods of in vitro digestion. The IVOMD values of green forages were determined using the RTT method only. Four ostrich diets and four green forages were digested in vitro using caecal liquor collected from ostriches slaughtered at the Norton Commercial ostrich producers (Copro) abattoir. The diets included a control diet of a concentrate and legume hay, silverleaf desmodium uncinatum (CN), and the three others consisting of a concentrate with either veld hay (VH), Katambora Rhodes grass hay (RG) or maize stover (MS). Lucerne (LU), Midmar Rye grass (MRG), Russian Comfrey (RC) and Kenyan White clover (KWC) were the green forages used. The forages were grown under the recommended management levels and were cut before flowering. There was no interaction (P > 0.05) between diet and method of digestion. The RTT gave higher (P < 0.01) IVOMD than the NTT method. However, both methods showed a similar trend in the digestion of the diets. The MS and RG diets had similar and greater (P < 0.05) IVOMD than the control and veld hay diets. The amounts of organic matter (OM) digested differed (P < 0.01) with either diet or forage type. Kenyan White clover and MRG had similar and greater (P < 0.05) IVOMD than LU and RC, which were not different (P > 0.05). The values of IVOMD in dry matter for the forages were:

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MRG > KWC > LU > RC, and all the differences were significant ($P < 0.05$). The study showed that the NTT method needs to be modified to suit hind gut fermenters and IVOMD can, therefore, be used to rank the nutritive value of ostrich feeds.

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Keywords: Ostrich diets; Forages; In vitro; Tilley and Terry

1. Introduction

To obtain a useful feed value, it is necessary to determine digestibility. Laboratory analysis of feeds may include measuring in vitro digestion. In vitro methods are much easier to perform and cheaper than conventional digestibility trials which are also time consuming. Biological methods, based on laboratory cultures of rumen microorganisms, have provided valuable information and a useful system of evaluation of feeds for ruminants (Van Der Meer, 1989). One such biological method is that of Tilley and Terry (1963), which mimics what happens in the gut of ruminants. Ruminants start with fermentation in the rumen before gastric digestion and absorption in the small intestine (Swart, 1988). Conversely, in hind gut fermenters such as ostriches and geese, diets are first exposed to the normal process of gastric digestion and intestinal absorption prior to microbial fermentation in the caeca/colon. Microbial fermentation yields volatile fatty acids, which are utilised for metabolism instead of glucose (Van Soest, 1982). Although in vitro techniques have been used widely for the evaluation of ruminant feeds, such methods were not available for the evaluation of non-ruminant feeds. The technique of Tilley and Terry (1963), which depends on the use of live rumen micro-organisms, has worked well in the evaluation of ruminant feeds such as forages. Use of caecal contents as a source of micro-organisms to evaluate ostrich feeds using a similar procedure but modified to suit hind gut fermenters such as ostriches may be useful. The ability of the ostrich to digest its food, especially the cheaper components which are green forages and conserved roughages, is important in relation to describing systems of feeding which are economically viable. This is particularly important in the Zimbabwean industry where gross margins in ostrich enterprises are declining and the sale of live birds is becoming less important compared to sale of meat and leather (Dzama et al., 1995).

The objective of the current study was to compare in vitro organic matter digestibility (IVOMD) using two methods (i.e. normal and reverse modified Tilley and Terry) of diets and green forages commonly fed to ostriches and to estimate the energy values of the latter.

2. Materials and methods

2.1. Feed samples

Four different ostrich diets were used. These were a conventional diet, that is a control diet (CN) of concentrate and a legume hay (*silverleaf desmodium*) and a concentrate with a poor quality roughage of either veld hay (VH), Katambora Rhodes grass hay (RG) or maize stover (MS). The details on ingredient composition of the diets were reported by Nheta
et al. (1997). In addition, four green forages, Lucerne (LU), Midmar Rye grass (MRG), Russian Comfrey (RC) and Kenyan White clover (KWC), grown under similar conditions were collected from the University of Zimbabwe farm and Veterinary Research Laboratory for in vitro digestibility determination. The green forages were harvested using a pair of scissors and chopped into small pieces of approximately 10 mm long. The small pieces were submerged into liquid nitrogen while still on site. They were freeze dried overnight at a pressure of $10^{-1}$ mbar and a temperature of $-50^\circ$C. Freeze drying was carried out to retain the essential characteristics of the original material such as chemical composition, appearance and colour.

Both the ostrich diets and green forages were ground to pass through a 1 mm screen. Representative samples of the forages were dried at 105 $^\circ$C overnight in a convection oven for dry matter determination and organic matter (OM) content was determined by further burning the dried samples in a muffle furnace and subtracting the ash component (AOAC, 1990).

2.2. Chemical analyses

The ostrich diets and green forages were analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to Van Soest et al. (1991). Crude protein (CP) was determined using the Kjedhal procedure (AOAC, 1990, Code 954.01).

2.3. Collection of caecal liquor

Thermoflasks were filled with distilled water (39 $^\circ$C) and left overnight in an incubator set at 39 $^\circ$C in order to maintain the incubation temperature. Caecal contents were collected from ostriches slaughtered at the Norton Copro abattoir situated 40 km west of Harare. Digesta samples from the caecum were emptied soon after evisceration, which took place approximately 40 min from stunning. These samples were then emptied into the warmed thermoflasks and flushed with nitrogen to maintain an anaerobic condition. On arrival at the University Laboratory some live caecal microbes were obtained by straining caecal contents through a muslin cloth to give caecal liquor.

2.4. In vitro organic matter digestibility

In vitro organic matter digestibility of the ostrich diets was determined using two methods. The first method was the normal modified Tilley and Terry technique (NTT) based on Tilley and Terry (1963). The NTT method mimics the digestion process in ruminants, which starts with fermentation in the rumen before gastric digestion and absorption in the small intestine. A second method, the reverse modified Tilley and Terry technique (RTT) was set up to simulate what happens in the gut of an ostrich. In ostriches, diets are firstly exposed to the normal process of gastric digestion and intestinal absorption prior to microbial fermentation in the caecum/colon. Since the NTT method underestimated the in vitro organic matter digestibility of the ostrich diets, the green forages were digested using the RTT method only.
2.4.1. Normal modified Tilley and Terry technique (NTT)

A buffer solution saturated with carbon dioxide (CO₂) to pH 6.8–6.9 was prepared and kept at a temperature of 39 °C. Feed samples (0.15 g) were weighed using a balance and put into fermentation flasks. Aliquots (20 ml) of caecal liquor were measured into each flask and 30 ml buffer solution was added and excess air above the liquor was displaced with nitrogen in order to create an anaerobic condition. The samples were placed in an incubator set at 39 °C for 48 h. The samples were filtered through sintered glass crucibles at the end of the first incubation period and 60 ml of acid pepsin added to each flask. The samples were incubated at 39 °C for a further 48 h. Shaking was done manually in the morning and evening for both stages.

2.4.2. Reverse modified Tillev and Terry technique (RTT)

The RTT method involved two stages of incubation. The first incubation stage was done with acid pepsin for 48 h. The second incubation stage over a further 48 h was carried out using caecal liquor instead of acid pepsin and the procedure was similar to the first stage of the NTT technique.

At the end of the second incubation period in both methods, samples were filtered through sintered glass crucibles and dried overnight in a convection oven at 105 °C. The dried sample residues and representative feed samples were incinerated in a muffle furnace in order to determine the indigestible organic matter (IDOM) and organic matter content, respectively. The in vitro digestibility of the diets and green forages was obtained by difference between the organic matter content of feed samples and the indigestible organic matter.

2.5. In vitro organic matter digestibility coefficients in dry matter of green forages

The in vitro organic matter digestibility coefficients in dry matter (D-values) were used to compare the four green forages.

2.6. Statistical analysis

Chemical composition of green forages and their respective D-values were compared using the general linear model procedure of SAS (1990). The model used to compare IVOMD of the ostrich diets contained method of digestion, diet and method × diet (2 × 4) interaction, whilst that used for green forages contained the forage type effects only.

3. Results

3.1. Chemical analysis

The chemical composition of the ostrich diets and the four green forages are shown in Tables 1 and 2, respectively. There were no significant differences (P > 0.05) in amounts of CP between the CN, RG and MS diets. The VH diet had the lowest (P < 0.05) level of CP. The amounts of ADF and ADL in RG and MS were similar, but significantly lower (P < 0.05) than those found in the CN and VH diets. All the green forages differed (P < 0.05)
Table 1
Crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) of ostrich diets containing four different roughages

<table>
<thead>
<tr>
<th>Diet</th>
<th>Chemical constituent (g kg(^{-1}) DM)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP</td>
<td>NDF</td>
<td>ADF</td>
<td>ADL</td>
</tr>
<tr>
<td>Control</td>
<td>159.3(^{a})</td>
<td>497.2</td>
<td>322.3(^{a})</td>
<td>113.3(^{a})</td>
</tr>
<tr>
<td>Veld hay</td>
<td>138.2(^{b})</td>
<td>474.4</td>
<td>282.4(^{a})</td>
<td>102.1(^{a})</td>
</tr>
<tr>
<td>Rhodes grass</td>
<td>151.1(^{a})</td>
<td>506.4</td>
<td>159.2(^{b})</td>
<td>51.2(^{b})</td>
</tr>
<tr>
<td>Maize stover</td>
<td>158.4(^{a})</td>
<td>456.1</td>
<td>153.2(^{b})</td>
<td>64.5(^{b})</td>
</tr>
<tr>
<td>SE</td>
<td>0.42</td>
<td>0.63</td>
<td>0.63</td>
<td>0.53</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Superscripts \(^{a}\) and \(^{b}\) denote significant differences in columns \((P < 0.05)\).

in their amounts of crude protein. Crude protein content was highest for LU (313.0 g kg\(^{-1}\)) and least for MRG (191.0 g kg\(^{-1}\)). There was a wide range (188.0–459.0 g kg\(^{-1}\)) in cell wall contents. Midmar Rye grass had the highest \((P < 0.05)\) cell wall contents. Russian Comfrey had the least \((P < 0.05)\) organic matter content and there were no differences \((P > 0.05)\) between LU, MRG and KWC.

3.2. In vitro organic matter digestibility and D-values

In vitro organic matter digestibility values of the ostrich diets are shown in Table 3 whereas those for green forages and their respective D-values are shown in Table 4. There was no interaction \((P > 0.05)\) between diet and method of digestion on digestibility coefficients of the diets. The RTT method gave higher \((P < 0.01)\) IVOMD values than the NTT method. Organic matter digestibilities, however, showed a similar trend of digestion in both methods. The MS and RG diets had a similar and greater \((P < 0.05)\) IVOMD than the CN and VH diets in each method of digestion. There was a negative relationship between lignin content of the diets and in vitro digestibility, as shown by the following equation: digestibility coefficient = 1.04 – 0.003 lignin (g kg\(^{-1}\) DM) \((R^2 = 0.87; P < 0.05)\).

The IVOMD differed \((P < 0.01)\) with the forage type. Kenyan White clover and MRG had similar and greater \((P < 0.05)\) digestibility than LU and RC. Lucerne had a numerically

Table 2
Crude protein (CP), neutral detergent fibre (NDF) and organic matter (OM) content of green forages fed to ostriches

<table>
<thead>
<tr>
<th>Forage</th>
<th>Chemical constituent (g kg(^{-1}) DM)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP</td>
<td>NDF</td>
<td>OM</td>
</tr>
<tr>
<td>Lucerne</td>
<td>313.5(^{a})</td>
<td>207.0(^{a})</td>
<td>900.1(^{a})</td>
</tr>
<tr>
<td>Midmar Rye grass</td>
<td>191.1(^{b})</td>
<td>459.6(^{c})</td>
<td>910.3(^{d})</td>
</tr>
<tr>
<td>Russian Comfrey</td>
<td>293.2(^{a})</td>
<td>188.1(^{b})</td>
<td>720.2(^{b})</td>
</tr>
<tr>
<td>Kenyan White clover</td>
<td>258.8(^{a})</td>
<td>197.0(^{b})</td>
<td>895.4(^{a})</td>
</tr>
<tr>
<td>SE</td>
<td>0.40</td>
<td>0.34</td>
<td>1.15</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Superscripts a–d denote significant differences in columns \((P < 0.05)\).
Table 3
Organic matter digestibility coefficients of ostrich diets containing different roughages using the normal (NTT) and reverse Tilley and Terry (RTT) methods of digestion

<table>
<thead>
<tr>
<th>Diet</th>
<th>Method of digestion</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NTT</td>
<td>RTT</td>
</tr>
<tr>
<td>Control</td>
<td>0.57 ± 0.018</td>
<td>0.62 ± 0.027</td>
</tr>
<tr>
<td>Veld hay</td>
<td>0.60 ± 0.019</td>
<td>0.72 ± 0.022</td>
</tr>
<tr>
<td>Rhodes grass</td>
<td>0.77 ± 0.019</td>
<td>0.84 ± 0.019</td>
</tr>
<tr>
<td>Maize stover</td>
<td>0.79 ± 0.017</td>
<td>0.84 ± 0.024</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.; NS: not significant (P > 0.05). Superscripts a–d denote significant differences.

* P < 0.05.
** P < 0.01.

Table 4
Organic matter digestibility (OMD) coefficients and in vitro organic matter digestibility in dry matter (D-values) of green forages using the reverse Tilley and Terry (RTT) method of digestion

<table>
<thead>
<tr>
<th>Forage</th>
<th>OMD</th>
<th>D-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucerne</td>
<td>0.80 ± 0.013</td>
<td>0.72 ± 0.012</td>
</tr>
<tr>
<td>Midmar Rye grass</td>
<td>0.86 ± 0.014</td>
<td>0.78 ± 0.013</td>
</tr>
<tr>
<td>Russian Comfrey</td>
<td>0.77 ± 0.014</td>
<td>0.56 ± 0.010</td>
</tr>
<tr>
<td>Kenyan White clover</td>
<td>0.84 ± 0.014</td>
<td>0.75 ± 0.012</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. Superscripts a–d denote significant differences within columns (P > 0.05).

higher IVOMD than Russian Comfrey although the difference did not attain significance (P > 0.05). The D-values for all the green forages were significantly different (P < 0.05), giving a pattern of MRG > KWC > LU > RC.

4. Discussion

4.1. Chemical composition and IVOMD of green forages

The variation in the chemical composition of the green forages indicated a wide range of forage quality. Organic matter content was high for LU, MRG and KWC but low for RC, probably due to its high silica content (Van Soest, 1982), resulting in a low nutritional value. The high amounts of CP and OM indicate that the green forages were of good quality. Midmar Rye grass, which had the highest contents of NDF and OM, also had the highest IVOMD. The observation implies that MRG contains cell wall contents, which are highly digestible. Although, the OM contents between MRG and LU were similar, Lucerne had the highest CP content. The observation that MRG had a higher IVOMD than LU could suggest that the CP in LU was not readily accessible to the microbes. It is interesting to note that RC and LU, which had the highest CP levels, had lower IVOMD than both MRG and KWC. This is probably due to two reasons. Firstly, it is probable that the RC and LU forages could
have supplied nitrogen in excess of the microbial requirements thereby reducing dietary fermentation. Secondly, the CP could have been bound and therefore not easily available.

The IVOMD values gave an indication that LU has a considerable amount of organic matter, which is not digestible. It can also be noted that although MRG had high cell wall contents, it was highly digestible as evidenced by its high D-value. It can, therefore, be concluded that although there is a wide range of green forages available for feeding ostriches, they differ in the amounts of digestible organic matter as indicated by the different D-values. These differences may imply differences in the nutritional values of the green forages.

4.1.1. In vitro organic matter digestibility of the ostrich diets

Differences in IVOMD of the ostrich diets could be explained by the ADF and ADL content of the diets (Nheta et al., 1997). These two fibre fractions showed a negative relationship with IVOMD, which agree with the findings of Pehrson and Faber (1994). Although the CN diet contained a concentrate plus legume, it had a lower DM digestibility than the VH, RG and MS diets that contained poor quality roughages. This unexpected result was probably due to the fact that the legume, silverleaf desmodium, is highly lignified and the protein is bound and, therefore, less digestible (Van Soest et al., 1991). The trend of IVOMD of the ostrich diets agrees with the in vivo DM digestibility values reported by Brooks and Urness (1984) and Nheta et al. (1997). The observation that the MS and RG diets had greater IVOMD than the CN and VH diets also agrees with the in vivo NDF digestibility (Nheta et al., 1997). The lower IVOMD for VH and CN diets suggest that both veld hay and silverleaf desmodium were of poor quality. The MS and RG diets, which had the highest digestibilities, had nearly half the contents of ADF and ADL compared with the VH and CN diets. The similarities in the patterns of in vitro and in vivo digestibilities could, therefore, suggest that the former can be used in evaluating the nutritive value of ostrich feeds.

The differences that were obtained in IVOMD for the ostrich diets between the two methods of analysis indicate that the NTT method tended to give a lower OMD than the RTT method. These lower IVOMD obtained using the NTT method show that the second stage of incubation with the caecal liquor played an important role in the digestion of the diets. The NTT method, which starts with fermentation with caecal liquor, because of the high concentrate fraction, rapidly produces fermentation acids, such as propionate. The build up of the acids reduces pH, which in turn, lowers microbial activity, which favours a pH range of 6.2–6.5 (Swart, 1988). It is probable that most of the readily soluble carbohydrates and proteins are digested during the first stage of incubation of the RTT method. The removal of these excess substrates from the concentrate fraction may create a suitable environment for microbial activity, which results in increased digestion of fibre. Although hydrolysis of carbohydrates and fats were not considered in this trial, the relative ranking in in vitro digestibility of CN < VH < RG < MS was similar to that of in vivo digestibilities reported earlier using growing birds (Nheta et al., 1997). The trend that was obtained in DM and NDF digestibilities, using the same four ostrich diets, were similar to the IVOMD obtained in the current study. For example, the in vivo DM digestible component for CN, VH, RG and MS was 659, 697, 732 and 803 g kg$^{-1}$ DM, respectively. In vitro organic matter digestibility determination can, therefore, be used to rank nutritive value of ostrich feeds.
Since the NTT method underestimated IVOMD, it can be concluded that the Tilley and Terry (1963) technique for ruminants needs to be modified to have a better assessment of diets containing a concentrate and roughage portion for ostriches.

5. Conclusion

Since IVOMD for the ostrich diets were lower in the normal than the reverse method of modified Tilley and Terry (1963), it can be concluded that the in vitro techniques used for ruminants needs to be modified to suit ostriches, which are hind gut fermenters. Organic matter digestibilities showed an almost similar trend of digestion in both methods. The IVOMD determination can be used to rank the nutritive value of different ostrich feeds. The variation in the chemical composition of the green forages indicated a wide range of forage quality. The different D-values for the green forages indicated a difference in their nutritive values. Therefore, organic matter digestibility in ostrich chicks depends on the type of green forage added to the ostrich starter concentrate diet. The findings in this study could imply that there is a wide range of forages that can be used in ostrich feeding.

Acknowledgements

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References
